CH2MHILL

Steve Costa
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11 July 2001

Mr. Carl Goldstein
Office of Pacific Islands and Native American Programs
Environmental Protection Agency
75 Hawthorne Street
San Francisco, CA 94105

Mr. Peter Peshut
American Samoa Environmental Protection Agency
American Samoa Government
P.O. Box 368A
Pago Pago, American Samoa96799

Enclosed is the required report for the bioassay test results for the March 2001 effluent sampling for Joint Cannery Outfall in American Samoa. The sampling and analysis were carried out without major problems, but the following items are of interest:

- The bioassay laboratory that had performed all of the previous tests went out of business shortly before the sampling and an alternative laboratory was selected for this test.

- The alternate test organism used for this test, *Mysidopsis bahia,* has been reclassified and is now referred to as *Americamysis bahia.*

- The laboratory inadvertently neglected to aerate the renewal concentrations used at 48-hours, and the apparent sudden increase in toxicity is undoubtedly overstated because of DO depression in the higher concentrations at this point. The results are provided for both exposure times in the report. However, the results are consistent with previous tests, and are not of concern, even for the 96-hour test results.

Please call us if you have any questions or comments on the enclosed report,
Sincerely,

Steven L. Costa

Cc: Jim Cox, COS International; Herman Gebauer, COS; Brett Ransby, COS; John Brown, Heinz; Phil Thirkel, StarKist Samoa; Joe Carney, StarKist Samoa; David Wilson, CH2M HILL.

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TECHNICAL MEMORANDUM

BIOASSAY TESTING – JOINT CANNERY OUTFALL EFFLUENT MARCH 2001 SAMPLING

Prepared For: StarKist Samoa (NPDES Permit AS0000019)
COS Samoa Packing (NPDES Permit AS0000027)

Prepared By: Steve Costa
Karen Glatzel

Date: 11 July 2001

Distribution: Carl Goldstein
United States Environmental Protection Agency, Region 9
Peter Peshut
American Samoa Environmental Protection Agency

Purpose

This memorandum presents the results of the bioassay testing of the Joint Cannery Outfall effluent sample that was collected in March 2001. The testing is required by the NPDES Permits that became effective in January 2001. This is the first required semiannual test required by the current permits and the fifteenth semiannual test conducted since testing for the Joint Cannery Outfall began in 1993.

Study Objectives

Section D.1 of the StarKist Samoa and COS Samoa Packing NPDES Permits requires that semiannual definitive acute bioassays (96-hour static bioassays) be conducted on the cannery effluent. The purpose of these tests is to determine whether, and at what effluent concentration, acute toxicity may be detected for the effluent.

Study Approach

U.S. EPA has conducted a number of reviews of the effluent sampling, analysis, and bioassay tests conducted in the past. All comments from U.S. EPA have been incorporated into either the Standard Operating Procedures (SOP) or have been incorporated into the procedures used by the laboratory doing the test and have been documented in previous reports.

The permit conditions require that the bioassay tests be conducted with the white shrimp, *Penaeus vannami* (postlarvae). In the event *Penaeus vannami* is not available at the time of the tests, the permit specifies that a substitute species, *Mysidopsis bahia,* may be used. The classification of *Mysidopsis bahia* has recently been changed to *Americamysis bahia* (see Attachment I). For the March 2001 sampling, *Penaeus vannami* was not available and *Americamysis bahia* was used.

Effluent samples were collected from the StarKist Samoa and COS Samoa Packing facilities as 24-hour composite samples. The effluent acute bioassay test was conducted using a combined composite effluent sample made up from the effluent samples from both canneries, as allowed by the permit condition. This combined effluent bioassay is representative of the wastewater discharged from the joint cannery outfall to Pago Pago Harbor.

Effluent Sampling Methods

Between 0900 on 21 March 2001 and 0600 on 22 March 2001, 24-hour flow-weighted composite samples of final effluent were collected from both the StarKist Samoa and COS Samoa Packing effluent discharges. Samples were collected from the established effluent sampling sites. Detailed sampling procedures are described in the established SOP for cannery effluent sampling.

A total of eight grab samples were collected into pre-cleaned 1-gallon plastic cubitainers at each plant. Samples were collected at approximately three-hour intervals over a 24-hour period. The samples were stored on ice until the completion of the 24-hour sampling period. After all samples were collected a flow-proportioned composite sample was prepared. The grab sample collection times, effluent flow rates, and the relative effluent flow volumes calculated from plant flow records are summarized in Table 1. The relative effluent flow volumes were used to prepare the final composite sample, which was used to fill the sample container shipped to the laboratory for testing.

A 5-gallon cubitainer containing the composite sample was packed on ice in an ice chest for shipment to the laboratory. A chain-of-custody form for the sample was completed and sealed into a zip-lock bag and taped inside the lid of the ice chest. The sample was shipped as checked baggage from Pago Pago to Honolulu and then to Seattle, WA and then repacked on ice and shipped via Federal Express to the testing laboratory. The testing laboratory received the sample on 26 March 2001. The chain-of-custody form is provided in Attachment II.

Table 1
StarKist Samoa and COS Samoa Packing
24-hour Composite Effluent Sample for Bioassay Testing
March 2001

Grab Sample Number	COS Samoa Packing		StarKist Samoa		COS Samoa Packing Percent of Total Flow	StarKist Samoa Percent of Total Flow
	Sampling Date and Time	Effluent Flow Rate (mgd)	Sampling Date and Time	Effluent Flow Rate (mgd)		
1	21 Mar 2001 0900	1.00	21 Mar 2001 0900	1.60	4.1	6.6
2	1200	0.92	1200	1.67	3.8	6.9
3	1500	0.96	1500	1.82	4.0	7.5
4	1800	1.24	1800	2.49	5.1	10.3
5	2100	1.02	2100	2.22	4.2	9.2
6	22 Mar 2001 0000	0.84	22 Mar 2001 0000	2.11	3.5	8.7
7	0300	1.18	0300	2.10	4.9	8.7
8	0600	0.88	0600	2.16	3.6	8.9
Total		8.04		16.17	33.2	66.8
Mean		1.01		2.02		

Bioassay Testing Procedures

EnviroSystems, Inc. in Hampton, New Hampshire conducted the bioassay tests[1]. The testing procedures and results of the bioassay tests are provided in the Laboratory report included as Attachment III. This report summarizes the 96-hour acute bioassay test conducted with reference to U.S. EPA document Methods for Measuring the Acute Toxicity of Effluents to Freshwater and Marine Organisms

[1] Advanced Biological Testing conducted all previous bioassay testing. This laboratory ceased doing business shortly before the March effluent sampling and EnviroSystems was selected to continue the bioassay testing program.

(EPA/600/4-90/027F), August 1993, as the source of methods for conducting the test. The bioassay test was conducted considering and including U.S. EPA's comments on previous bioassay tests, as documented in previous reports.

The test organisms were ≤ 5 days old and the test temperature was held at 20 ±1°C and salinity was adjusted to 27 ppt. Because of the demonstrated potential for a lethal immediate dissolved oxygen demand (IDOD), discussed and documented in previous technical memoranda describing the first two bioassay tests conducted in 1993, each bioassay test chamber was continuously aerated during the bioassay tests to maintain adequate levels of dissolved oxygen (DO). The test was renewed with sample at 48 hours and resulted in apparent sudden and substantial toxicity. However, this is attributed to the drop in DO because the renewal concentrations were not aerated prior to the renewal[2] (see Attachment I and III).

Bioassay tests were carried out for effluent concentrations of 50, 25, 12.5, 6.25, and 3.1% as vol:vol dilutions in seawater. Water quality was monitored daily and parameters measured included DO, pH, salinity, and temperature. Total residual chlorine and ammonia were measured. Additionally, a reference toxicant test using sodium dodecyl sulfonate (SDS) was conducted and results were within one standard deviation of the established laboratory mean.

Results

The results of the bioassay tests are summarized as follows:

***Americamysis bahia* Effluent Bioassay.** All results from the bioassay tests are included in Attachment II. The results of the mysid bioassay tests indicate the 96-hour LC_{50} for the effluent tested was 13.8 percent. The No Observable Effects Concentration (NOEC) for the 96-hour bioassay was 12.5 percent and the Least Observable Effects Concentration (LOEC) was 25 percent. However, as discussed above, the toxicity is probably overstated because of the drop in DO to less than 1.5 mg/l for the 25 percent test concentration at the time of test concentration renewal. Results on a daily basis are summarized in Table 2.

***Americamysis bahia* Reference Toxicant Bioassay.** The reference toxicant test had an LC_{50} of 23.4 mg/l. The laboratory mean is 19.9±4.34 mg/l (based

[2] This was an inadvertent oversight by the new laboratory and will be corrected for future tests.

on 122 tests). The test data falls within one standard deviation of the laboratory reference mean, indicating normal sensitivity.

Table 2
StarKist Samoa and COS Samoa Packing
Combined Effluent Bioassay Results
March 2001 Sampling

Exposure Time	Parameter		
	LC_{50}	NOEC	LOEC
24 hours	45.97%	25%	50%
48 hours	39.08%	25%	50%
72 hours	14.93%	12.5%	25%
96 hours	13.81%	12.5%	25%

Discussion

Table 3 summarizes the results of the effluent bioassay tests for the samples collected in the March 2001 sampling compared to the previous bioassay tests. The LC_{50}, NOEC and LOEC are within the range obtained from previous reports where *Mysidopsis bahia* was used in place of *Penaeus vannami.*

Conclusions

The bioassay tests for the Joint Cannery Outfall effluent for March 2001 do not indicate effluent toxicity levels to be of concern. As discussed in the previous bioassay test reports on the effluent, the time scale of the mixing of the effluent with the receiving water is on the order of minutes to seconds to achieve dilutions that will eliminate possible toxic effects as reflected by the bioassay results. For example, an NOEC of 12.5%, which was observed in March 2001, corresponds to a dilution of 8:1, which is achieved within a second and within 1-meter of the discharge point. The discharge is located in about 180 feet of water and the effluent toxicity tests indicate that the discharge is diluted to non-toxic levels immediately after discharge and well within the initial dilution plume.

Table 3
StarKist Samoa and COS Samoa Packing
Combined Effluent Bioassay Results

Date	Species	Parameters		
		LC 50	NOEC	LOEC
2/93	*Penaeus vannami*	4.8%[1]	3.1%	6.25%
10/93	*Penaeus vannami*	15.67%	3.1%	6.25%
2/94	*Penaeus vannami*	15.76%	<1.6%	1.6%
10/94	*Mysidopsis bahia*[2]	31.2%	25%	50%
3/95	*Penaeus vannami*	14.8%	6.25%	12.5%
3/95	*Mysidopsis bahia*[3]	10.8%	6.25%	12.5%
2/96	*Penaeus vannami*	>50%	>50%	>50%
2/96	*Mysidopsis bahia*[3]	28.36%	12.5%	25%
3/96	*Penaeus vannami*	44.4%	25%	50%
11/96	*Penaeus vannami*	7.11%	3.1%	6.25%
03/97	*Penaeus vannami*	39.36%	12.5%	25%
09/97	*Penaeus vannami*[4]	12.3%	6.25%	12.5%
06/98	*Mysidopsis bahia*[2]	17.2%	6.25%	12.5%
11/98	*Mysidopsis bahia*[2]	15%	6.25%	12.5%
02/00	*Mysidopsis bahia*[2]	20%	6.25%	12.5%
08/00	*Mysidopsis bahia*[2]	17.1%	3.1%	6.25%
03/01	*Americamysis bahia*[5]	13.8%	12.5%	25%

[1]The February 1993 samples were not aerated until after the first day of the test. For subsequent tests the samples were aerated for the entire duration of the tests.

[2]*Mysidopsis bahia* used as substitutes because *Penaeus vannami* not available: as directed and approved by U. S. EPA.

[3]*Mysidopsis bahia* used in addition to *Penaeus vannami* as described in text of technical memorandums reporting test results. Only one species is required by the permit conditions.

[4]Stage 1 (3 mm) *Penaeus vannami* were used for testing because older Stage 7 and 8 (8-10 mm) *Penaeus vannami* were not available.

[5]*Mysidopsis bahia* renamed *Americamysis bahia.* Results indicate increased toxicity because of low DO in renewal concentrations as renewal water was not aerated prior to use

ATTACHMENT I

Letter from ESI
Regarding organism name change
and bioassay test deviations

ESI

EnviroSystems, Inc.
P.O. Box 778, One Lafayette Road
Hampton, N.H. 03843-0778
(603) 926-3345 • (603) 926-3521 Fax
E-mail ESI @ www.envirosystems.com

May 21, 2001

Mr. Steve Costa / Ms. Karen Glatzel
CH2M Hill, Incorporated
P.O. Box 1238
Trinidad, California 95570-1238

Dear Mr. Costa:

It came to our attention last year that the classification of the mysid shrimp, *Mysidopsis bahia*, had been changed to *Americamysis bahia* [1]. This change in no way affects any of the test results we have reported. The change brings our reports up to date with the currently accepted nomenclature for this species.

As we discussed, the testing we reported for an effluent sample collected from the Samoa Joint Cannery Outfall during March 2001, experienced considerable toxicity between 48 and 72 hours. Please note that the most likely cause for this toxicity was low dissolved oxygen. The test was renewed with sample at 48 hours. The renewal concentrations were not aerated prior to adding the mysid shrimp; therefore the organisms were subjected to extremely low dissolved oxygen concentration for a short time. Once the renewal took place, all of the concentrations were placed back on air. The survival and LC-50 results at 24, 48, 72 and 96 hours were noted in the executive summary (page 2) and in Table 1 (page 6) of the original report.

If you have any questions or concerns related to these matters please free to call me or Kenneth Simon.

Sincerely,

EnviroSystems, Incorporated

Linnea Hauthaway
Laboratory Manager

[1] SETAC. December 1999. *Environmental Toxicology and Chemistry; An International Journal*, Volume 18, Number 12, pp.2888-2893. Pensacola, Florida

ATTACHMENT II

Chain-of Custody

CH2MHILL Applied Sciences Lab
CHAIN OF CUSTODY RECORD
AND AGREEMENT TO PERFORM SERVICES

CVO 2300 NW Walnut Boulevard
Corvallis, OR 97330-3638
(541) 752-4271 FAX (541) 752-0276

COC # _____

Project #	Purchase Order #

Project Name: JCO 2001 NONTRADEWIND

Company Name: CH2M HILL

Project Manager or Contact & Phone #	Report Copy to:
STEVE COSTA 707-677-0123	SAME

Requested Completion Date:	Site ID	Sample Disposal: Dispose ☒ Return ☐

Sampling Date	Sampling Time	Type COMP	Type GRAB	Matrix WATER	Matrix SOIL	Matrix AIR	CLIENT SAMPLE ID (9 CHARACTERS)	LAB QC	TOTAL # OF CONTAINERS	Requested Analytical Method # — ACUTE BIOASSAY w/ MYSIDOPSIS BAHIA	Preservative — N/O
3/21		X		X			C1-NT		2	X	

Requested Analytical Method #

Preservative

THIS AREA FOR LAB USE ONLY

Lab #	Page	of

Lab PM	Custody Review

Log In	LIMS Verification

pH	Custody Seals Y N
	Ice Y N

QC Level 1 2 3 Other _____

Cooler Temperature

Alternate Description	Lab ID
Dilution Series	
100, 50, 25, 12.5, 6.25	
REPORT LC50	
& NOEC	

Relinquished By	Date/Time	Received By — Empty Bottles	Date/Time
Sampled By and Title: JIM CRAWFORD (Please sign and print name)	Date/Time 3/21/01	Relinquished By: JIM CRAWFORD (Please sign and print name)	Date/Time 3/21/01
Received By (Please sign and print name)	Date/Time	Relinquished By (Please sign and print name)	Date/Time
Received By (Please sign and print name)	Date/Time	Shipped Via: UPS (Fed-Ex) Other	Shipping #

Special Instructions:

Instructions and Agreement [illegible]

ATTACHMENT III

ESI Laboratory Report

TOXICOLOGICAL EVALUATION OF A TREATED EFFLUENT: BIOMONITORING SUPPORT FOR A NPDES PERMIT MARCH 2001

American Samoa Joint Cannery Outfall

Prepared For

CH2M Hill, Incorporated
P.O. Box 1238
Trinidad, California 95570-1238

By

EnviroSystems, Incorporated
One Lafayette Road
Hampton, New Hampshire 03842

March 2001
Reference Number CH2M9406-01-03

STUDY NUMBER 9406

EXECUTIVE SUMMARY

The following summarizes the results of acute exposure bioassays performed from March 27-31, 2001 in support of the NPDES biomonitoring requirements of the American Samoa Joint Cannery Outfall. The 96 hour acute definitive assays were conducted using the marine species, *Americamysis bahia.*

Acute Toxicity Evaluation

Species	Exposure	LC-50	NOEC	LOEC
Americamysis bahia	24-Hours	45.97%	25%	50%
	48-Hours	39.08%	25%	50%
	72-Hours	14.93%	12.5%	25%
	96-Hours	13.81%	12.5%	25%

COMMENTS:

Results reflect test concentrations after salinity adjustment. See Section 2.3.

Authorized Signature: _____ 5/2/07

President ~ EnviroSystems, Incorporated — Date

TOXICOLOGICAL EVALUATION OF A TREATED EFFLUENT: BIOMONITORING SUPPORT FOR A NPDES PERMIT MARCH 2001

American Samoa Joint Cannery Outfall

1.0 INTRODUCTION

Acute toxicity tests involve preparing a series of concentrations by diluting effluent with control water. Groups of test animals are exposed to each effluent concentration and a control for a specified period. In acute tests, mortality data for each concentration are used to calculate (by regression) the median lethal concentration, or LC-50, defined as the effluent concentration which kills half of the test animals. Samples with high LC-50 values are less likely to cause significant environmental impact. The acute no observed effect concentration (NOEC) and lowest observed effect concentration (LOEC) document the highest and lowest effluent concentrations that have no impact and a significant impact on the test species, respectively.

This report presents the results of acute toxicity tests conducted on an effluent sample collected from the American Samoa Joint Cannery Outfall. Testing was based on programs and protocols developed by the US EPA (1993) and involved conducting 96 hour acute static renewal toxicity tests with the marine species, *Americamysis bahia*. Testing was performed at EnviroSystems, Incorporated (ESI), Hampton, New Hampshire.

2.0 MATERIALS AND METHODS

2.1 General Methods

Toxicological and analytical protocols used in this program follow procedures primarily designed by the EPA to provide standard approaches for the evaluation of toxicological effects of discharges on aquatic organisms, and for the analysis of water samples. See Section 4.0 for a list of references.

2.2 Test Species

A. bahia, ≤5 days old, were from cultures maintained by Aquatic Research Organisms, Incorporated of Hampton, New Hampshire. Test organisms were transferred to test chambers by large bore pipet, minimizing the amount of water added to test solutions.

2.3 Effluent and Dilution Water

The effluent sample used in the assay was identified as "01-NT". Sample collection information is provided in Table 4. Upon receipt, the unused sample portion was stored at 4°C. All sample material used in the assay was warmed to 20±1°C prior to preparing test solutions. Total residual chlorine (TRC) was measured using amperometric titration (MDL 0.05 mg/L). As the effluent sample contained <0.05 mg/L TRC dechlorination with sodium thiosulfate was not required (EPA 1993). Subsamples of the undiluted effluent sample were collected for ammonia analysis when the sample arrived and again prior to renewal. At arrival, the effluent sample had a salinity of 13‰. Salinity of the effluent was increased to 27‰ by the addition of 36 grams of artificial sea salts to 2000 mL of effluent. Test concentrations for the assays were 50%, 25%, 12.5%, 6.25% and 3.1% effluent with dilution water control.

The dilution water used in this assay was obtained by EnviroSystems from its sea water system. The water is pumped from the Hampton Estuary on the flood tide, filtered through a high volume sand filter and stored in 3000 gallon polyethylene tanks. The water is classified as Class A waters by the State of New Hampshire and has been used for culture of test organisms for over 20 years. Sea water used in the assay had a salinity of 26‰ and a TRC of <0.05 mg/L.

2.4 Acute Toxicity Tests

The 96 hour acute static renewal toxicity tests were conducted at 20±1°C with a photoperiod of 16:8 hours light:dark. Test chambers for the acute assays were 250 mL glass beakers containing 200 mL test solution in each of 5 replicates with 10 organisms/replicate. Survival, dissolved oxygen, pH, salinity and temperature were measured daily in all replicates. Test solutions were renewed after 48 hours exposure with effluent from the start sample. Mysid shrimp were fed <24 hour old brine shrimp on a daily basis.

2.5 Data Analysis

Survival data at 24 hour intervals were analyzed to assess toxicity using a program developed by Stephan (1982) which computes LC-50 values using the Spearman-Karber, Probit, binomial and Moving Average computation methods. If survival in the highest test concentration was >50%, LC-50 values were obtained by direct observation of the raw data. The NOEC was determined as the highest test concentration which caused no significant mortality while the LOEC was determined as the lowest concentration that did cause significant mortality.

2.6 Quality Control

As part of the laboratory quality control program, standard reference toxicant assays are conducted on a regular basis for each test species. These results provide relative health and response data while allowing for comparison with historic data sets. A forty-eight hour acute reference toxicant assay was performed with *A. bahia* on March 27, 2001. Results of this assay was within one standard deviation of its respective historic mean. See Table 2 for details.

3.0 RESULTS

Results of the acute exposure bioassay conducted using the mysid shrimp are summarized in Table 1. A summary of reference toxicant data for the test species is presented in Table 2. Effluent and dilution water characteristics are presented in Table 3. Sample collection information is provided in Table 4. Table 5 provides a summary of historic data associated with the discharge. Support data are included in Appendix A.

3.1 Acute Toxicity Test - *Americamysis bahia*

There was 100% survival in laboratory diluent control after 96 hours exposure. These results are an indication of healthy test organisms and that the dilution water had no adverse impact on the outcome of the assay.

Table 1 provides a summary of the acute exposure data and results.

3.2 Summary

The salinity adjusted effluent sample for the American Samoa Joint Cannery site exhibited signs of acute toxicity to the mysid shrimp, *Americamysis bahia*, during the 96 hour exposure period.

4.0 LITERATURE CITED

APHA. 1995. *Standard Methods for the Examination of Water and Wastewater*, 19th edition. Washington D.C.

Stephan, C. 1982. Documentation for Computing LC-50 Values with a Mini Computer. Unpublished.

US EPA. 1993. *Methods for Measuring the Acute Toxicity of Effluents to Freshwater and Marine Organisms*. Fourth Edition. EPA/600/4-90/027F.

US EPA. 2000. *Attachment G: NPDES Whole Effluent Toxicity Testing, Monitoring and Reporting Tips and Common Pitfalls.* Dated December 2000. US EPA Region I Offices, Boston, Massachusetts.

TABLE 1. Summary of Acute Evaluation Results. American Samoa Joint Cannery Outfall Effluent Evaluation. March 2001.

Concentration % Effluent	Exposure	Replicates A	B	C	D	E	Mean	Standard Deviation	Coefficient of Variation
Lab Control	Start	10	10	10	10	10			
	24-Hours	10	10	10	10	10	100%	0.000	0.00%
	48-Hours	10	10	10	10	10	100%	0.000	0.00%
	72 Hours	10	10	10	10	10	100%	0.000	0.00%
	96-Hours	10	10	10	10	10	100%	0.000	0.00%
3.1%	24-Hours	10	10	10	10	10	100%	0.000	0.00%
	48-Hours	10	10	10	10	10	100%	0.000	0.00%
	72 Hours	10	10	10	10	10	100%	0.000	0.00%
	96-Hours	9	10	10	10	8	94%	0.894	95.15%
6.25%	24-Hours	10	10	10	10	10	100%	0.000	0.00%
	48-Hours	10	10	10	10	10	100%	0.000	0.00%
	72 Hours	10	10	10	10	10	100%	0.000	0.00%
	96-Hours	10	9	10	10	10	98%	0.447	45.63%
12.5%	24-Hours	9	10	10	10	10	98%	0.447	45.63%
	48-Hours	9	9	10	10	9	94%	0.548	58.27%
	72 Hours	0	9	10	10	9	76%	4.278	562.88%
	96-Hours	0	9	10	10	10	78%	4.382	561.77%
25%	24-Hours	10	10	10	10	10	100%	0.000	0.00%
	48-Hours	10	10	10	9	10	98%	0.447	45.63%
	72 Hours	1	0	0	0	0	2%	0.447	2236.07%
	96-Hours	1	0	0	0	0	2%	0.447	0.00%
50%	24-Hours	10	2	0	4	4	40%	3.742	935.41%
	48-Hours	5	0	0	4	0	18%	2.490	1383.32%
	72 Hours	0	0	0	0	0	0%	0.000	0.00%
	96-Hours	0	0	0	0	0	0%	0.000	0.00%

SUMMARY OF ENDPOINTS

Exposure Period	LC-50	NOEC	LOEC
24-Hours	45.97% (25-50)	25.0%	50.0%
48-Hours	39.08% (36-42)	25.0%	50.0%
72-Hours	14.93% (14-16)	12.5%	25.0%
96-Hours	13.81% (12-15)	12.5%	25.0%

TABLE 2. Summary of Reference Toxicant Data. American Samoa Joint Cannery Outfall Effluent Evaluation. March 2001.

Concentrations Expressed as mg/L Sodium Dodecyl Sulfate

Species	Date	LC-50	Historic Mean	Number of Tests	±1 STD Deviation	±2 STD Deviation
A. bahia	03/27/01	23.4	19.9	122	4.34	8.68

TABLE 3. Summary of Effluent and Diluent Characteristics. American Samoa Joint Cannery Outfall Effluent Evaluation. March 2001.

Parameter	Units	EFFLUENT	DILUENT
Salinity - on Arrival	‰	13	26
After Salinity Adjustment ‡	‰	27	-
pH - on Arrival	SU	6.67	7.62
After Salinity Adjustment ‡	SU	7.09	-
TRC	mg/L	<0.05	<0.05
Dissolved Oxygen	mg/L	3.5	7.3
Ammonia - at Start	mg/L as N	78.02	<0.10
Unionized Ammonia	mg/L as N	0.15	-
Ammonia - at 48 Hours	mg/L as N	97.71	<0.10
Unionized Ammonia	mg/L as N	0.19	-

TABLE 4. Summary of Sample Collection Information. American Samoa Joint Cannery Outfall Effluent Evaluation. March 2001.

Sample Description	Type	Collection Date	Collection Time	Receipt Date	Receipt Time	Arrival Temp °C
EFFLUENT	Comp	03/21/01	ND	03/26/01	ND	ND

COMMENTS:
‡ - Recorded in the 50% effluent concentration, not 100% salinity adjusted effluent.
ND - No data for this parameter was recorded.

TABLE 5. Summary of StarKist Samoa and COS Samoa Packing Combined Effluent Bioassay Results. American Samoa Joint Cannery Outfall Effluent Evaluation. March 2001.

Date	Species	96-Hour Endpoints		
		LC-50	NOEC	LOEC
02/93[1]	*Penaeus vannami*	4.8%	3.1%	6.25%
10/93[1]	*Penaeus vannami*	15.67%	3.1%	6.25%
02/94[1]	*Penaeus vannami*	15.76%	<1.6%	1.6%
10/94[1]	*Americamysis bahia*	31.2%	25.0%	50.0%
03/95[1]	*Penaeus vannami*	14.8%	6.25%	12.5%
03/95[1]	*Americamysis bahia*	10.8%	6.25%	12.5%
02/96[1]	*Penaeus vannami*	>50.0%	>50.0%	>50.0%
03/96[1]	*Penaeus vannami*	44.4%	25.0%	50.0%
11/96[1]	*Penaeus vannami*	7.11%	3.1%	6.25%
03/97[1]	*Penaeus vannami*	39.36%	12.5%	25.0%
09/97[1]	*Penaeus vannami*	12.3%	6.25%	12.5%
06/98[1]	*Americamysis bahia*	17.2%	6.25%	12.5%
11/98[1]	*Americamysis bahia*	15.0%	6.25%	12.5%
02/00[1]	*Americamysis bahia*	20.0%	6.25%	12.5%
08/00[1]	*Americamysis bahia*	17.1%	3.1%	6.25%
03/01[2]	*Americamysis bahia*	13.81%	12.5%	25.0%

Notes:

1. Assays conducted by Advanced Biological Testing, Inc., Rohnert Park, California

2. Assay conducted by EnviroSystems, Inc., Hampton, New Hampshire

APPENDIX A

DATA SHEETS

STATISTICAL SUPPORT

Contents	Number of Pages
Methods Used in NPDES Permit Biomonitoring Testing	1
A. bahia Acute Bioassay Laboratory Bench Sheets	2
LC-50 Computation Printouts	8
A. bahia Organism Culture Sheet	1
Dilution Preparation Log	1
Water Quality Instruments Record Log	1
Sample Receipt Record	1
Chain of Custody	1
Certificate of NELAC Accreditation	2

METHODS USED IN NPDES PERMIT BIOMONITORING TESTING

Parameter	Method
Acute Exposure Bioassays	
Ceriodaphnia dubia, Daphnia pulex	EPA 600/4-90/027
Pimephales promelas	EPA 600/4-90/027
Americamysis bahia	EPA 600/4-90/027
Menidia beryllina, Cyprinodon variegatus	EPA 600/4-90/027
Chronic Exposure Bioassays	
Ceriodaphnia dubia	EPA 600/4-91/002, 1002.0
Pimephales promelas	EPA 600/4-91/002, 1000.0
Cyprinodon variegatus	EPA 600/4-91/003, 1004.0
Menidia beryllina	EPA 600/4-91/003, 1006.0
Arbacia punctulata	EPA 600/4-91/003, 1008.0
Champia parvula	EPA 600/4-91/003, 1009.0
Trace Metals:	
ICP Metals	EPA 200.7/SW 6010
Hardness	Standard Methods 20th Edition - Method 2340 B
Wet Chemistries:	
Alkalinity	Standard Methods 20th Edition - Method 310.1
Chlorine, Residual	Standard Methods 20th Edition - Method 4500CLD
Total Organic Carbon	Standard Methods 20th Edition - Method 5310.6
Specific Conductance	Standard Methods 20th Edition - Method 2510B
Nitrogen - Ammonia	Standard Methods 20th Edition - Method 4500NH3G
pH	Standard Methods 20th Edition - Method 4500H+B
Solids, Total (TS)	Standard Methods 20th Edition - Method 2540.B
Solids, Total Suspended (TSS)	Standard Methods 20th Edition - Method 2540D
Dissolved Oxygen	Standard Methods 20th Edition - Method 4500-O G

STUDY: 9406	SAMPLE RECEIVED: 3/27/01	"AS RECEIVED" EFFLUENT AND DILUENT CHEMISTRIES					
CLIENT: CH2M Hill	TEST ORGANISM: *A. bahia*		TRC	AMM 0 HR✖	AMM 48 HR✖	pH	DO	Salinity
SAMPLE: American Samoa	ORGANISM SUPPLIER: ARO	EFFLUENT	<0.05	SE	SE	6.67	3.5	13
DILUENT: LAB SALT	ORGANISM BATCH/AGE: 032701ABARO 5 days	Lab Salt DILUENT	<0.05	✓	SE	7.62	7.3	26

SALINITY ADJUSTMENT RECORD (IF APPLICABLE): 2000 ML EFFLUENT + 36 G SEA SALTS = 100% ACTUAL PERCENTAGE

CONC	REP	SURVIVAL					✦DISSOLVED OXYGEN (MG/L)✦						PH (SU)						TEMPERATURE (°C)						SALINITY (ppt)					
		0	24	48	72	96	0	24	48◊	48☆	72	96	0	24	48◊	48☆	72	96	0	24	48◊	48☆	72	96	0	24	48◊	48☆	72	96
LAB	A	10	10	10	10	10	7.3	7.3	7.5	7.3	7.5	7.5	7.64	8.11	7.86	7.70	7.85	7.89	21	21	20	21	20	19	26	27	27	26	26	27
	B	10	10	10	10	10	7.0	6.5	7.6	7.1	7.4	7.6	7.66	7.81	7.99	7.64	7.90	8.04	21	21	20	21	20	19	26	27	28	26	26	27
	C	10	10	10	10	10	7.0	6.4	7.7	7.2	7.5	7.6	7.67	8.11	8.03	7.64	7.93	8.09	21	21	20	21	20	19	26	27	28	26	27	28
	D	10	10	10	10	10	7.0	6.7	7.7	7.2	7.6	7.6	7.68	7.89	7.97	7.65	7.91	8.11	21	21	20	21	20	20	26	27	28	26	27	28
	E	10	10	10	10	10	6.9	6.3	7.7	7.3	7.9	7.6	7.68	7.91	8.0	7.67	7.90	8.12	21	21	20	21	20	20	26	27	28	26	26	27
3.1%	A	10	10	10	10	9	6.9	7.0	7.6	7.2	7.6	7.4	7.65	8.08	8.05	7.43	7.97	8.03	21	21	20	21	20	19	26	27	28	27	26	28
	B	10	10	10	10	10	6.9	7.2	7.6	7.1	7.7	7.5	7.63	8.14	8.04	7.40	7.95	8.10	21	21	20	21	20	19	26	27	29	27	26	28
	C	10	10	10	10	10	6.9	7.0	7.7	6.9	7.9	7.5	7.65	7.95	8.00	7.38	7.92	8.13	21	21	20	21	20	19	26	27	29	27	26	27
	D	10	10	10	10	10	6.9	7.2	7.7	6.6	7.9	7.6	7.64	8.08	8.02	7.41	7.94	8.15	21	21	20	21	20	19	26	27	28	27	27	27
	E	10	10	10	10	8	6.9	7.1	7.7	6.7	8.0	7.7	7.65	8.13	8.02	7.50	7.97	8.14	21	21	20	21	20	20	26	27	29	26	26	28
6.25%	A	10	10	10	10	10	6.7	7.1	7.7	6.6	7.9	7.5	7.58	8.13	8.01	7.55	7.98	8.15	21	21	20	21	20	19	26	27	29	26	26	28
	B	10	10	10	10	9	6.7	7.1	7.6	6.4	8.0	7.4	7.58	8.11	8.07	7.60	7.97	8.17	21	21	20	21	20	19	26	27	29	26	27	28
	C	10	10	10	10	10	6.7	7.2	7.6	6.4	7.9	7.4	7.55	8.15	8.08	7.58	7.98	8.20	21	21	20	21	20	19	26	27	29	26	27	28
	D	10	10	10	10	10	6.6	7.3	7.5	6.4	7.9	7.5	7.53	8.13	8.11	7.57	8.00	8.21	21	21	20	21	20	19	26	27	29	26	27	28
	E	10	10	10	10	10	6.7	7.3	7.6	6.4	7.9	7.6	7.54	8.06	8.18	7.71	8.00	8.16	21	21	20	21	20	19	26	27	29	26	27	27
DATE		3/27	3/28	3/29	3/30	3/31	3/27	3/28	3/29	3/29	3/30	3/31	3/27	3/28	3/29	3/29	3/30	3/31	3/27	3/28	3/29	3/29	3/30	3/31	3/27	3/28	3/29	3/29	3/30	3/31
TIME		1630	1540	1630	1500	1455	1600	1500	1500	1600	1500	1445	1600	1500	1500	1600	1500	1445	1600	1500	1500	1600	1500	1445	1600	1500	1500	1600	1500	1445
INITIALS		SE	SE	SE	✓	®	SE	SE	SE	SE	✓	®	SE	SE	SE	SE	✓	®	SE	SE	SE	SE	✓	®	SE	SE	SE	SE	✓	®
FED?		✓	✓	✓	✓																									

✖ - Pull on 50% effluent also.
< - "Old" water qualities (prior to renewal)

✦ - AERATE FROM START!
☆ - "New"water qualities (post renewal)

ACUTE BIOASSAY DATA SUMMARY

STUDY: 9406	SAMPLE RECEIVED: 3/27/01	"AS RECEIVED" EFFLUENT AND DILUENT CHEMISTRIES					
CLIENT: CH2M Hill	TEST ORGANISM: *A. bahia*		TRC	AMM 0 HR✖	AMM 48 HR✖	pH	DO	Salinity
SAMPLE: American Samoa	ORGANISM SUPPLIER: AQUO	EFFLUENT	See page 1 for details and salinity adjustment record.					
DILUENT: LAB SALT	ORGANISM BATCH/AGE: ≤ 5 days	DILUENT						

CONC	REP	SURVIVAL					✦DISSOLVED OXYGEN (MG/L)✦						PH (SU)						TEMPERATURE (°C)						SALINITY (ppt)					
		0	24	48	72	96	0	24	48◊	48☆	72	96	0	24	48◊	48☆	72	96	0	24	48◊	48☆	72	96	0	24	48◊	48☆	72	96
12.5%	A	10	9	9	0	—	6.5	7.0	7.4	4.8	5.1	—	7.47	8.11	8.11	7.77	7.56	—	21	21	20	21	20	—	26	27	28	26	26	27
	B	10	10	9	9	9	6.5	7.1	7.4	5.0	6.0	7.6	7.56	8.12	7.95	7.87	7.42	8.18	21	21	20	21	20	19	26	28	29	26	26	27
	C	10	10	10	10	10	6.5	7.0	7.4	4.5	6.1	7.6	7.48	8.14	8.08	7.93	8.05	8.22	21	21	20	21	20	19	26	28	29	26	26	27
	D	10	10	10	10	10	6.5	6.8	7.4	4.4	6.1	7.5	7.44	7.99	8.12	7.94	8.01	8.24	21	21	20	21	20	19	26	28	29	26	26	28
	E	10	10	9	9	10	6.4	6.9	7.4	4.9	6.4	7.5	7.45	8.10	7.13	7.95	8.01	8.23	21	21	20	21	20	19	26	28	29	26	26	27
25%	A	10	10	10	1	1	6.5	7.2	7.2	1.0	6.1	7.6	7.30	8.16	8.08	7.93	8.06	8.05	21	21	21	21	20	19	26	28	29	26	26	28
	B	10	10	10	0	—	6.3	7.0	7.3	1.3	6.0	—	7.28	8.11	8.10	7.91	8.03	—	21	21	21	21	20	—	27	28	29	26	26	—
	C	10	10	10	0	—	6.3	6.8	7.2	1.2	6.4	—	7.26	8.10	8.07	7.90	8.04	—	21	21	21	21	20	—	27	27	29	26	26	—
	D	10	10	9	0	—	6.0	6.7	7.1	1.4	6.7	—	7.25	8.09	8.09	7.91	8.08	—	21	21	21	21	20	—	27	27	29	26	26	—
	E	10	10	10	0	—	6.0	6.6	7.0	1.1	6.7	—	7.23	8.12	8.12	7.85	8.00	—	21	21	21	21	20	—	27	28	29	26	27	—
50%	A	10	10	5	0	—	5.2	6.7	7.4	1.1	6.7	—	7.11	8.13	8.21	7.82	8.09	—	20	21	21	21	20	—	27	28	29	26	27	—
	B	10	2	0	—	—	5.2	6.5	7.4	—	—	—	7.10	8.07	8.17	7.82	—	—	20	21	21	21	—	—	27	28	29	26	—	—
	C	10	0	—	—	—	5.2	6.4	7.3	—	—	—	7.10	8.08	8.16	7.81	—	—	20	21	21	21	—	—	27	28	29	26	—	—
	D	10	4	4	0	—	5.2	6.4	3.8	1.5	6.2	—	7.68	8.13	8.04	7.87	8.06	—	20	21	21	21	20	—	27	28	29	26	28	
	E	10	4	0	—	—	5.1	6.3	7.0	—	—	—	7.09	8.15	8.22	7.85	—	—	20	21	21	21	—	—	27	28	29	26	—	—
DATE		3/21	3/22	3/23	3/24	3/25	3/21	3/22	3/23	3/24	3/25	3/26	3/21	3/22	3/23	3/24	3/25	3/26	3/27	3/28	3/29	3/30	3/31	4/1	3/27	3/28	3/29	3/30	3/31	4/1
TIME		1634	1540	1500	1500	1455	1600	1500	1500	1600	1500	1445	1600	1500	1500	1600	1500	1445	1600	1500	1500	1600	1500	1445	1600	1500	1500	1600	1500	1445
INITIALS		SE	SE	SE	✓	(R)	SE	SE	SE	SE	✓	(R)	SE	SE	SE	SE	✓	(R)	SE	SE	SE	SE	✓	(R)	SE	SE	SE	SE	✓	(R)
FED?		✓	✓	✓	✓																									

✖ - Pull on 50% effluent also.
◊ - "Old" water qualities (prior to renewal)

✦ - AERATE FROM START!
☆ - "New" water qualities (post renewal)

* SE 3/29/01

****

STUDY NO.: 9406 ASSAY START: 03/27/01

SPECIES: Americamysis bahia EXPOSURE: 24 Hours

SAMPLE: American Somoa Joint Cannery Venture

****

At a confidence level of 95 percent, the binomial test shows that the LC50 is above 25

The usefulness of any LC50 calculated from this set of data is questionable because a concentration-effect relationship has not been demonstrated over a reasonable range (e.g., <37 to >63) of percent affected.
An approximate LC50 of 45.9688 is obtained by nonlinear interpolation between 25 and 50

----------RESULTS CALCULATED USING THE MOVING AVERAGE METHOD----------

The Moving Average Method cannot be used with this set of data because no span produces moving averages which bracket 50 percent dead and also uses two concentrations which have percent dead between 0 and 100 percent.

----------RESULTS CALCULATED USING THE PROBIT METHOD----------

Iterations	G	H	Chi-Square	Probability
7	3.491616	10.04893	30.14679	LESS THAN 0.001

As the probability associated with this value is <0.05, results should be used with caution.

Slope = 5.545136
95 Percent Confidence Limits =-4.816431 and 15.9067

LC50 = 47.04924
95 Percent Confidence Limits = 0 and +INFINITY

---------RESULTS CALCULATED USING THE TRIMMED SPEARMAN-KARBER METHOD-------

Conc.	Dose =Ln (Conc)	Percent Dead	Monotonic Rel. Freq.	Trimmed Rel. Freq.
50	3.912023	60	.6	.625
25	3.218876	0	.01	-.1125 *
12.5	2.525729	2	.01	-.1125 *
6.25	1.832581	0	0	-.125 *
3.1	1.131402	0	0	-.125 *

Alpha = 10 %
Groups trimmed and therefore not used in estimating LC50 are marked with an asterisk above.
LC50 = 9.596971
Estimated 95 Percent Confidence Limits
Lower: 7.895702 Upper: 11.66481
Variance estimate = 9.518831E-03
Variance may be underestimated because all data were outside trimmed rang

****

STUDY NO.: 9406 ASSAY START: 03/27/01

SPECIES: Americamysis bahia EXPOSURE: 24 Hours

SAMPLE: American Somoa Joint Cannery Venture

****

SUMMARY TABLE

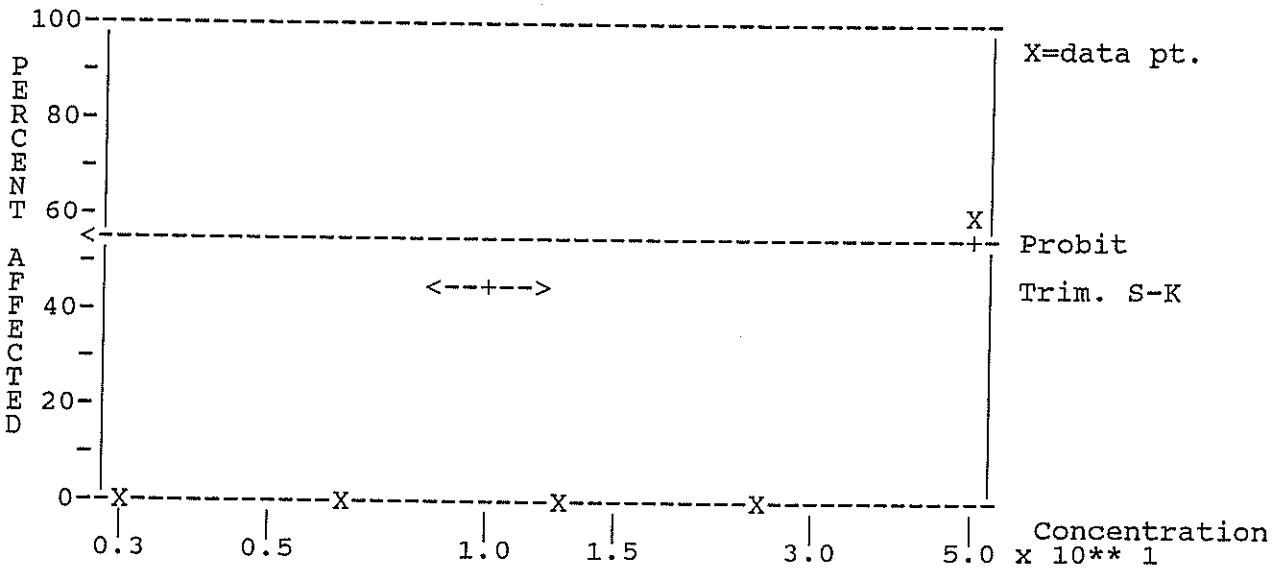
04-26-2001 09:15:26

Data:	Conc.	Exposed	Dead	Percent
	50	50	30	60
	25	50	0	0
	12.5	50	1	2
	6.25	50	0	0
	3.1	50	0	0

	LC50	Lower 95% Limit	Upper 95% Limit	
Probit Analysis	47.04924	0	0	
Moving Average	0	0	0	Span= 4
Spearman-Karber	9.596971	7.895702	11.66481	Alpha= 10 %
Binomial	45.9688			

Compare results with original data to see if they are reasonable.

****



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STUDY NO.: 9406 ASSAY START: 03/27/01

SPECIES: Americamysis bahia EXPOSURE: 48 Hours

SAMPLE: American Somoa Joint Venture Cannery Venture

****

The binomial test shows that 25 and 50 can be used as statistically sound, conservative 95 percent confidence limits because the actual confidence level associated with these limits is greater than 95 percent.

An approximate LC50 of 39.08408 is obtained by nonlinear interpolation between 25 and 50

----------RESULTS CALCULATED USING THE MOVING AVERAGE METHOD----------

Span	G	LC50	95 Percent Confidence Limits	
1	4.172688E-02	39.08407	36.40364	42.32988

An LC50 calculated using the Moving Average method may not be a very good estimate if the span is much less than the number of concentrations.

If any higher concentration produces a lower percent affected than a lower concentration, the confidence limits obtained by the Moving Average method will probably be too close.

----------RESULTS CALCULATED USING THE PROBIT METHOD----------

Iterations	G	H	Chi-Square	Probability
7	2.163003	9.647164	28.9415	LESS THAN 0.001

As the probability associated with this value is <0.05, results should be used with caution.

Slope = 5.065605
95 Percent Confidence Limits =-2.384457 and 12.51567

LC50 = 37.65236
95 Percent Confidence Limits = 0 and +INFINITY

---------RESULTS CALCULATED USING THE TRIMMED SPEARMAN-KARBER METHOD-------

Conc.	Dose =Ln (Conc)	Percent Dead	Monotonic Rel. Freq.	Trimmed Rel. Freq.
50	3.912023	82	.82	.9
25	3.218876	2	.04	-.075 *
12.5	2.525729	6	.04	-.075 *
6.25	1.832581	0	0	-.125 *
3.1	1.131402	0	0	-.125 *

Alpha = 10 %
Groups trimmed and therefore not used in estimating LC50 are marked with an asterisk above.
LC50 = 25.35302
Estimated 95 Percent Confidence Limits
Lower: 23.29619 Upper: 27.59144
Variance estimate = 1.789617E-03
Variance may be underestimated because all data were outside trimmed range.

****

STUDY NO.: 9406 ASSAY START: 03/27/01

SPECIES: Americamysis bahia EXPOSURE: 48 Hours

SAMPLE: American Somoa Joint Venture Cannery Venture

****

SUMMARY TABLE

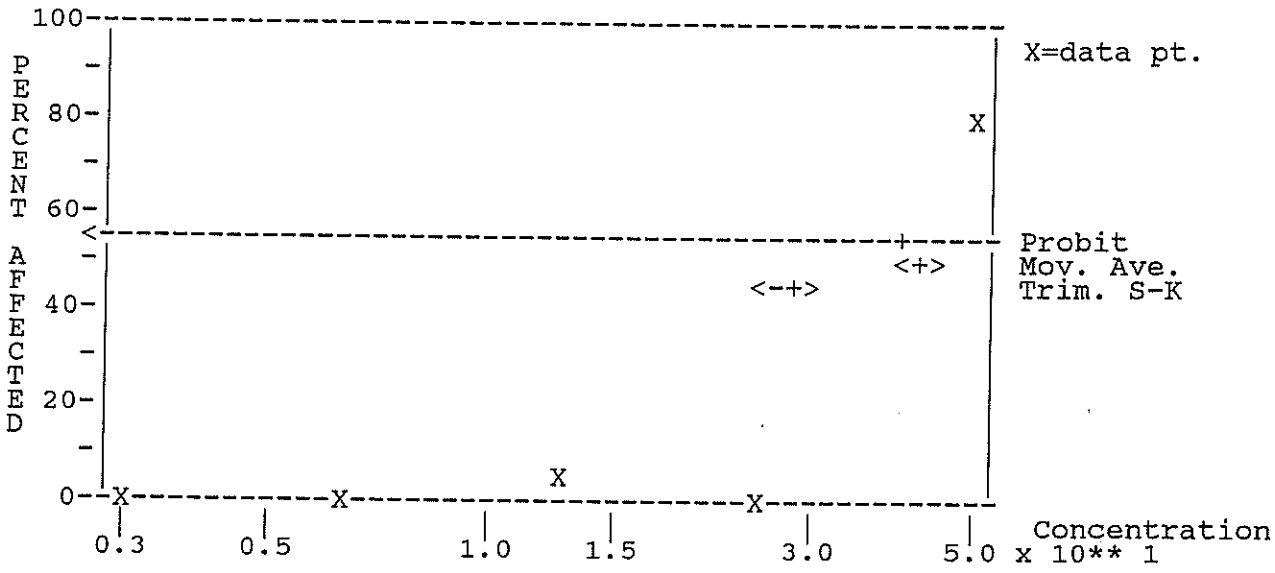
04-26-2001 09:18:49

Data: Conc.	Exposed	Dead	Percent
50	50	41	82
25	50	1	2
12.5	50	3	6
6.25	50	0	0
3.1	50	0	0

	LC50	Lower 95% Limit	Upper 95% Limit	
Probit Analysis	37.65236	0	0	
Moving Average	39.08407	36.40364	42.32988	Span= 1
Spearman-Karber	25.35302	23.29619	27.59144	Alpha= 10 %
Binomial	39.08408			

Compare results with original data to see if they are reasonable.

****



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STUDY NO.: 9406 ASSAY START: 03/27/01

SPECIES: Americamysis bahia EXPOSURE: 72 Hours

SAMPLE: American Somoa Joint Cannery Venture

****

The binomial test shows that 12.5 and 25 can be used as statistically sound, conservative 95 percent confidence limits because the actual confidence level associated with these limits is greater than 95 percent.

An approximate LC50 of 15.42128 is obtained by nonlinear interpolation between 12.5 and 25

----------RESULTS CALCULATED USING THE MOVING AVERAGE METHOD----------

Span	G	LC50	95 Percent Confidence Limits	
4	1.866134E-02	14.92571	13.16258	17.03956
3	1.859222E-02	14.93479	13.34701	16.60504
2	2.148203E-02	14.74734	13.49564	16.23254
1	4.877926E-02	15.42128	14.07079	16.66634

An LC50 calculated using the Moving Average method may not be a very good estimate if the span is much less than the number of concentrations.

----------RESULTS CALCULATED USING THE PROBIT METHOD----------

Iterations	G	H	Chi-Square	Probability
6	9.869112E-02	1	1.296616E-02	.9996089

Slope = 9.206671
95 Percent Confidence Limits = 6.314382 and 12.09896

LC50 = 14.92827
95 Percent Confidence Limits = 13.69102 and 16.4762

---------RESULTS CALCULATED USING THE TRIMMED SPEARMAN-KARBER METHOD-------

Conc.	Dose =Ln (Conc)	Percent Dead	Monotonic Rel. Freq.	Trimmed Rel. Freq.
50	3.912023	100	1	1
25	3.218876	98	.98	.98
12.5	2.525729	24	.24	.24
6.25	1.832581	0	0	0
3.1	1.131402	0	0	0

Alpha = 0 %
Groups trimmed and therefore not used in estimating LC50 are marked with an asterisk above.
LC50 = 15.17743
Estimated 95 Percent Confidence Limits
Lower: 13.89731 Upper: 16.57547
Variance estimate = 1.94103E-03

****

STUDY NO.: 9406 ASSAY START: 03/27/01

SPECIES: Americamysis bahia EXPOSURE: 72 Hours

SAMPLE: American Somoa Joint Cannery Venture

****

SUMMARY TABLE

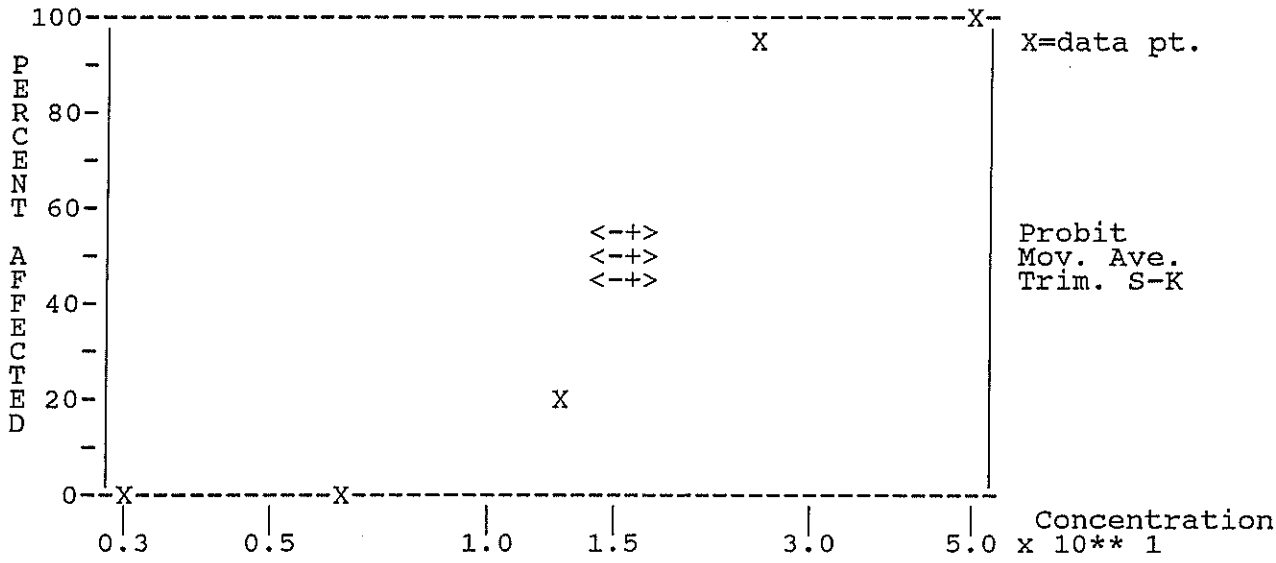
04-26-2001 09:24:25

Data: Conc.	Exposed	Dead	Percent
50	50	50	100
25	50	49	98
12.5	50	12	24
6.25	50	0	0
3.1	50	0	0

	LC50	Lower 95% Limit	Upper 95% Limit	
Probit Analysis	14.92827	13.69102	16.4762	
Moving Average	14.93479	13.34701	16.60504	Span= 3
Spearman-Karber	15.17743	13.89731	16.57547	Alpha= 0
Binomial	15.42128			

Compare results with original data to see if they are reasonable.

****



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STUDY NO.: 9406 ASSAY START: 03/27/01

SPECIES: Americamysis bahia EXPOSURE: 96 Hours

SAMPLE: American Somoa Joint Cannery Venture

****

The binomial test shows that 12.5 and 25 can be used as statistically sound, conservative 95 percent confidence limits because the actual confidence level associated with these limits is greater than 95 percent.

An approximate LC50 of 15.27239 is obtained by nonlinear interpolation between 12.5 and 25

----------RESULTS CALCULATED USING THE MOVING AVERAGE METHOD----------

Span	G	LC50	95 Percent Confidence Limits	
4	2.499335E-02	13.16138	11.37332	15.27085
3	2.148187E-02	13.80775	12.17234	15.4939
2	2.148204E-02	14.17089	12.97518	15.56229

An LC50 calculated using the Moving Average method may not be a very good estimate if the span is much less than the number of concentrations.

If any higher concentration produces a lower percent affected than a lower concentration, the confidence limits obtained by the Moving Average method will probably be too close.

----------RESULTS CALCULATED USING THE PROBIT METHOD----------

Iterations	G	H	Chi-Square	Probability
5	3.923429	31.57399	94.72198	LESS THAN 0.001

As the probability associated with this value is <0.05, results should be used with caution.

Slope = 4.417817
95 Percent Confidence Limits =-4.33284 and 13.16847

LC50 = 13.68908
95 Percent Confidence Limits = 0 and +INFINITY

Cannot calculate V1 component of variance.

---------RESULTS CALCULATED USING THE TRIMMED SPEARMAN-KARBER METHOD-------

Conc.	Dose =Ln (Conc)	Percent Dead	Monotonic Rel. Freq.	Trimmed Rel. Freq.
50	3.912023	100	1	1
25	3.218876	100	1	1
12.5	2.525729	22	.22	.22
6.25	1.832581	2	.04	.04
3.1	1.131402	6	.04	.04

Alpha = 0 %
Groups trimmed and therefore not used in estimating LC50 are marked with an asterisk above.
LC50 = 13.91049
No variance estimate could be made.

****

STUDY NO.: 9406 ASSAY START: 03/27/01

SPECIES: Americamysis bahia EXPOSURE: 96 Hours

SAMPLE: American Somoa Joint Cannery Venture

****

SUMMARY TABLE

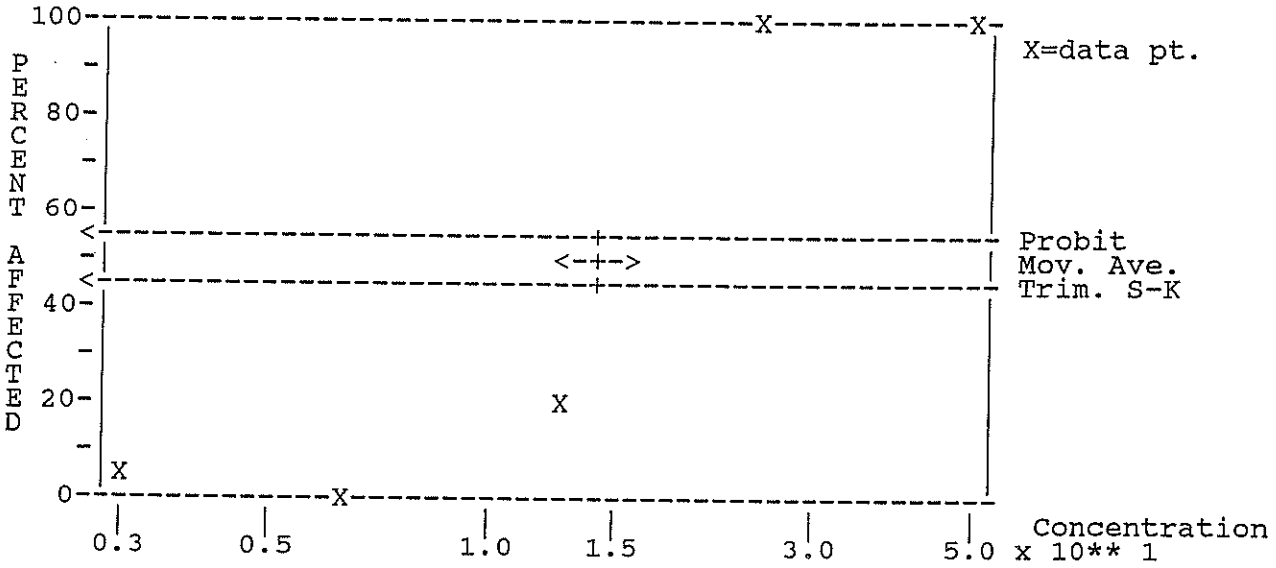
04-26-2001 09:27:16

Data: Conc.	Exposed	Dead	Percent
50	50	50	100
25	50	50	100
12.5	50	11	22
6.25	50	1	2
3.1	50	3	6

	LC50	Lower 95% Limit	Upper 95% Limit	
Probit Analysis	13.68908	0	0	
Moving Average	13.80775	12.17234	15.4939	Span= 3
Spearman-Karber	13.91049	0	0	Alpha= 0 %
Binomial	15.27239			

Compare results with original data to see if they are reasonable.

****



Lot # 032701ABARO

Aquatic Research Organisms

DATA SHEET

I. Organism History

Species: AMERICAMYSIS bahia

Source: Lab reared ✓ Hatchery reared _____ Field collected _____

Hatch date 3-22-01 Receipt date _____

Lot number 032201MS Strain _____

Brood Origination Florida

II. Water Quality

Temperature 25 °C Salinity ≈28 ppt DO —

pH 7.8 Hardness ✓ ppm

III. Culture Conditions

System: Recirc

Diet: Flake Food _____ Phytoplankton _____ Trout Chow ✓

Brine Shrimp ✓ Rotifers _____ Other Encap. Shrimp Diet

Prophylactic Treatments: _____

Comments: _____

_____

IV. Shipping Information

Client: ESI # of Organisms: 420 +

Carrier: _____ Date Shipped: 3-27-01

Biologist: Mark Rosenquist

1 - 800 - 927 - 1650

PO Box 1271 • One Lafayette Road • Hampton, NH 03842 • (603) 926-1650

PREPARATION OF DILUTIONS

STUDY: 9406	CLIENT: CH2M HILL - American Samoa
SPECIES: *A. bahia*	

Diluent: Lab Salt	Day: 0		Day: 2	
	Sample: EoA		Sample: EcA	
Concentration	Vol. Eff.	Final Vol	Vol. Eff.	Final Vol
LAB	0	1000	0	800
3.1%	31	↓	24.8	↓
6.25%	62.5		50	
12.5%	125		100	
25%	250		200	
50%	500	↓	400	↓

HRS	Date	Time	Initials
0	3/27	1535	SE
48	3/28	1530	SE

Comments:

Amms. pulled on 100% Eff and 50% Eff conc.

RECORD OF METERS USED FOR WATER QUALITY MEASUREMENTS

STUDY: 9406	CLIENT: CH2M HILL - American Samoa					
WATER QUALITIES - A. bahia						
HOURS:	0	24	48 - old	48 - new	72	96
Water Quality Station #	1	1	1	1	1	1
Initials	SE	SE	SE	SE	[illegible]	[illegible]
Date	3·27·01	3·28·01	3. 29. 01	3'29'01	3·30·01	3/31/01

Water Quality Station #1		Water Quality Station #2		COMMENTS
DO meter #	1821	DO meter #		
DO probe #	23	DO probe #		
pH meter #	1097	pH meter #		
pH probe #	25	pH probe #		
S/C meter #	95E30	S/C meter #		
S/C probe #	1	S/C probe #		
Salinity meter #	95E30	Salinity meter #		

CH2M HILL Applied Sciences Lab
CHAIN OF CUSTODY RECORD
AND AGREEMENT TO PERFORM SERVICES

CVO 2300 NW Walnut Boulevard
Corvallis, OR 97330-3538
(541) 752-4271 FAX (541) 752-0276

COC # ____

Field	Entry
Project #	
Purchase Order #	
Project Name	JCO 2001 NONTRADEWIND
Company Name	CH2M HILL
Project Manager or Contact & Phone #	STEVE COSTA 707-677-0123
Report Copy to:	SAME
Requested Completion Date:	
Site ID	
Sample Disposal:	Dispose ☒ Return ☐

Requested Analytical Method #: ACUTE BIOASSAY w/ MYSIDOPSIS BAHIA

Preservative: NO

Date	Time	Type: COMP	Type: GRAB	Matrix: WATER	Matrix: SOIL	Matrix: AIR	CLIENT SAMPLE ID (9 CHARACTERS)	LAB QC	TOTAL # OF CONTAINERS	ACUTE BIOASSAY w/ MYSIDOPSIS BAHIA
3/21		X		X			01-NT		2	X

THIS AREA FOR LAB USE ONLY

Lab #	Page ___ of ___
Lab PM	Custody Review
Log In	LIMS Verification
pH	Custody Seals Y N
	Ice Y N
QC Level 1 2 3 Other ____	
Cooler Temperature	

Alternate Description	Lab ID
Dilution Series	
100, 50, 25, 12.5, 6.25	
REPORT LC50	
& NOEC	

Relinquished By	Date/Time	Received By / Empty Bottles	Date/Time
Sampled By and Title (Please sign and print name) JIM CRAWFORD	Date/Time 3/21/01	Relinquished By (Please sign and print name) JIM CRAWFORD	Date/Time 3/25/01
Received By (Please sign and print name)	Date/Time	Relinquished By (Please sign and print name)	Date/Time
Received By (Please sign and print name)	Date/Time	Shipped Via UPS (Fed-Ex) Other ____	Shipping #

Special Instructions:

Instructions and Agreement Provisions on Reverse Side

DISTRIBUTION: Original - LAB Yellow - LAB Pink - Client